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HPTLC FINGERPRINT PROFILE AND STANDARDIZATION OF *CYAMOPSIS TETRA-GONOLOBA* (L.) TAUB.

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Keywords:

Cyamopsis tetragonoloba, Phytochemical, HPTLC profiling, Microbial activity, Physicochemical evaluation, Microscopical evaluation

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ABSTRACT: *Cyamopsis tetragonoloba* belongs to the family Fabaceae and has notable medicinal properties as it is used as a digestive aid. Guar Gum, which is a high molecular weight carbohydrate with multiple industrial applications, is also obtained from this plant. Methanolic extracts of *C. tetragonoloba* were obtained through reflux extraction to perform further experiments. Various experimental tests were carried out to understand the physicochemical, antimicrobial, and fluorescence properties of *C. tetragonoloba*. HPTLC and phytochemical profiles were also obtained. Preliminary phytochemical analysis showed the presence of alkaloids, flavonoids, saponins, steroids and triterpenes. Microscopy of the sample powder showed the presence of macrosclereids and thickened xylem vessels. The maximum antimicrobial activity of *C. tetragonoloba* was seen at a sample concentration of 10 µL where the activity was more than standard against *Escherichia coli* and *Aspergillus niger*. HPTLC profiling showed 7 bands each in 5 lanes of increasing concentration and a pattern unique to *C. tetragonoloba*.

INTRODUCTION: India has a primitive history of traditional medicines. Indian traditional medicines originated from numerous systems include Folk literature, Siddha, Unani, Ayurveda, Naturopathy and Homeopathy. One such plant in this regard is *Cyamopsis tetragonoloba* (L.) Taub., commonly known as guar bean or cluster bean. It is a leguminous plant that belongs to the Fabaceae family.

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Guar is a coarse, upright, bushy, drought-resistant summer annual plant ranging from 2-4 feet in height. It has pointed, saw-toothed, trifoliate leaves, small purplish white flowers borne along the axis of a spikelet, and hairy pods 3-4 inches long in clusters. There are both dwarf and tall cultivars. Guar flowers are self-pollinating.

A mature unopened bud starts out white, and then changes to a light pink as petals begin to open [1]. Cluster beans are typically grown as a rainfed crop in arid and semi-arid climates [2]. It also has high salinity resistance and requires 200 to 375mm annual rainfall with plenty sunshine [3]. High temperature and lower relative humidity have been associated to increased seed output and gum content in the plant [4].

Ascorbic acid, condensed tannins, galactomannans, gallic acid, gentisic acid, caffeine, p-coumaric acid, astragalin, P-hydroxycinamyl, and coniferyl alcohol are all present in the leaves and pods. The amount of polyphenols in the plant's seeds and leaves varies according to its maturation level. Gum derived from the seed's endosperm contains a polysaccharide called galactomannan that has a mannose backbone and a galactose side group, and it's mannopyranose units are connected by a 1–4 linkage. It has excellent water thickening ability and only a small amount is required to achieve sufficient viscosity. Hence it is widely used in food, cosmetics, and textile industries in the form of guar gum powder [5]. Another crucial ingredient that contributes to the guar's 85% composition is orthodihydrics [6].

According to Ayurveda, the *C. tetragonoloba* plant is used to lessen fire and can also be used as a digestive aid, tonic, galactagogue, and as a remedy for dyspepsia, anorexia, agalactia, hyetalopia, and vitiated kapha and pitta conditions. Guar gum is a laxative made from a plant's seed fiber that also aids in treating diabetes, obesity, IBS, diarrhea, and hardening of the arteries (atherosclerosis) [7]. Leaves are used to treat asthma and night blindness [8]. Seeds are used as a trypsin inhibitor, anti-oxidant, anti-inflammatory, laxative, and in the treatment of sprains, swellings, and arthritis. Boiling seeds are used as a poultice for swellings caused by broken bones, an enlarged liver, the plague, and head swellings [9]. In conclusion, *C. tetragonoloba* and its valuable seed component, guar gum, have become pivotal resources in various industries due to their unique properties. Therefore, the aim of this paper is to study and analyze the microbiological, HPTLC, pharmacognostic and phytochemical profile of *Cyamopsis tetragonoloba* (L.) Taub., to assess and understand it's various applications and benefits.

MATERIALS AND METHODOLOGY:

Collection of Sample: In this study, samples of *Cyamopsis tetragonoloba* were collected from a local market at Bangalore. The robust mature pods were targeted for our study, following a random sampling approach. The pods were dried in the sunlight, later in the oven for 3-5 days and then ground it in order to prepare a powder of the pods. The powder was stored at room temperature for further analysis. The collected sample was identified and authenticated by Flora of Presidency of Madras and JCB Herbarium with voucher specimen number- CS1684 [10-11].

Organoleptic Evaluation: Organoleptic evaluation is a qualitative method based on the sample's morphological characteristics where it is evaluated based on sight, smell, taste, touch and color. It is the conclusion drawn from studies that resulted due to impressions on organs of senses [12-13].

Physicochemical Evaluation:

Determination of Total Ash: Total ash is the amount of ash obtained after the sample has been incinerated and is devoid of carbon. It usually contains phosphates, carbonates, silicates and silica. An empty crucible was weighed after heating in the muffle furnace at 500 °C for 1 hour. 2 g of powdered sample was weighed and transferred into the dried and cooled empty crucible which was then placed in the muffle furnace with the temperature setting at 600 °C. After 4 hours, the crucible was removed from the furnace and cooled by placing in a desiccator for 30 min after which the following readings were noted [14].

$$\text{Total Ash \%} = B\text{-}C / A \times 100$$

Where, A = Weight of sample (in grams), B = Weight of crucible + contents after drying (in grams), C = Weight of empty crucible (in grams).

Determination of Acid Insoluble Ash: Total ash when treated with HCl reacts with materials to form soluble salts and insoluble residues consisting of mainly silica and acid insoluble ash. 25 mL of 2M HCl was added to the crucible containing the total ash obtained and covered with a watch glass. This setup was kept on a hot plate to boil gently for 5 min. The insoluble matter was filtered using a clean filter paper which was then rinsed repeatedly with hot water until it was neutral or free from acid. This filter paper containing the insoluble matter was then transferred into the original crucible. The crucible was dried on hot plate and placed in the muffle furnace at a temperature setting of 500 °C. After 4 hours, the crucible was removed and cooled by placing in a desiccator for 30 min after which the following readings were noted [14].

$$\text{Acid Insoluble Ash \%} = B\text{-}C / A \times 100$$

Where, A = Weight of sample (in grams), B = Weight of crucible + contents after drying (in grams), C = Weight of empty crucible (in grams).

Determination of Alcohol Soluble Extract: 4 g of powdered plant material was weighed and macerated with 100 mL of 90% ethanol in a conical flask for 24 hours while shaking the contents frequently in the first 6 hours. Thereafter, the contents of the flask were rapidly filtered while taking precautions against loss of ethanol.

An empty petri plate was weighed and the contents obtained after filtration was poured into it and placed on the hot water bath till all the liquid component had evaporated. The petri plate was then dried by placing it in a hot air oven at a temperature setting of 105 °C, followed by placing it in a desiccator for 30 min. The following readings were noted [15].

Alcohol Soluble Extractive value % = B-C / A × 4 × 100

Where, A = Weight of plant material (in grams), B = Weight of petri plate + residue, C = Weight of empty petri plate.

Determination of Water Soluble Extract: 4 g of powdered plant material was weighed and macerated with 100 mL of chloroform water in a conical flask for 24 hours while shaking the contents frequently in the first 6 hours. Thereafter, the contents of the flask were rapidly filtered. An empty petri plate was weighed and the contents obtained after filtration was poured into it and placed on the hot water bath till all the liquid component had evaporated. The petri plate was then dried by placing it in a hot air oven at a temperature setting of 105 °C, followed by placing it in a desiccator for 30 min. The following readings were noted [15].

Water Soluble Extractive value % = B-C / A × 4 × 100

Where, A = Weight of plant material (in grams), B = Weight of petri plate + residue, C = Weight of empty petri plate.

Preliminary Phytochemical Evaluation: Phytochemicals, also known as secondary metabolites, are biologically active compounds found in plants like alkaloids, flavonoids, saponins, steroids, triterpenes, phenols, tannins *etc*., these phytochemicals are found in various parts of the plant like leaves, bark, seeds, flowers and roots. The methanolic extracts were subjected to various chemical tests to detect the chemical constituents present in them. Different tests were performed for different constituents i.e., alkaloids (Dragendorff's test, Mayer's test), flavonoids (Shinoda test), saponins (Froth test), steroids (Liebermann-Burchard's test, Salkowski test), triterpenes (Liebermann-Burchard's test, Salkowski test) and phenols (Ferric chloride test) [16-18].

HPTLC Studies: Sample solutions were applied to the Silica gel 60 F254 (E. Merck) precoated TLC plates as sharp bands by means of Aspire automatic sample applicator.

The spots were dried in a current of air. Chromatography was carried out in a glass chamber (Aspire). The mobile phase (Toluene: Ethyl acetate (8:2 v/v)) was poured into a twin trough glass chamber. Whole assembly was left to equilibrate and pre-saturate for 30 min.

The plate was then developed until the solvent front had travelled at a distance of 80 mm above the base of the silica plate at 20 ºC and 50% relative humidity. The plate was visualized for detection by observing it under UV light (254 nm) and at long UV (366 nm). Then the derivatization was carried with 10% H_2SO_4 solution. The densitometric scan was drawn using Just TLC software attached to Aspire HPTLC [19].

Microscopical Evaluation:

Powder Microscopy: It is a quality control method used for medicinal plants to study the specific microscopic characters using different staining reagents. The powdered plant material was completely immersed in 10% HCl overnight. The sample was then filtered and rinsed with distilled water the next day. The soaked sample was completely drained of excess water, stained with safranin and observed in Magnus MLX Plus microscope under 4X magnification followed by 10X, 40X, and 100X [20].

Powder Fluorescence: Powdered sample when treated with different chemical reagents gives characteristic color when visualized under UV light. On adding 1-2 drops of nitrocellulose on the

sample and observed in UV Transilluminator, a change in color is noticed [21].

Fluorescence Analysis: The powdered sample of *C. tetragonoloba* was treated with various chemicals like water, concentrated HNO_3, H_2SO_4 and HCl, methanol, petroleum ether, hexane, chloroform, and ethanol. The powdered materials gave different colors with different chemicals when observed under UV light of short and long wavelengths [21].

Microbial Limit Test: Microbial testing is carried out for the detection of microorganisms in a product. We check for the amount of microbial activity in the sample chosen. The suitable media- Nutrient Agar, Potato Dextrose Agar and peptone water was prepared and sterilized. Serial dilution was performed by adding 10 g of powdered sample in 90 mL NaCl saline (10^{-1}). 9 mL of NaCl saline was poured in a series of 9 test tubes. 1 mL of sample from the first dilution was taken and mixed with the contents of second test tube (10^{-2}).

This process was continued for up to 4 dilutions. 0.1 or 1 mL of sample was taken from each dilution and poured into the respective petri plates and inoculated with the media by pour plate method. Incubate the bacterial plates with Nutrient Agar at 37 °C for 24-48 hours and fungal plates with the Potato Dextrose Agar at room temperature for 3-5 days. Observe and count for bacterial and fungal colonies with the help of a digital colony counter after the incubation period [22-23].

Microbial Activity:

Antibacterial Activity: The *in-vitro* antibacterial activity test was conducted against two different bacteria- *Escherichia coli* and *Bacillus cereus*, as these bacteria multiply rapidly and show results quickly. The sample was prepared by adding 40 mg of plant extract in 10 mL DMSO. The *in-vitro* antibacterial activity test was conducted against two different bacteria- *Escherichia coli* and *Bacillus cereus*, as these bacteria multiply rapidly and show results quickly. The sample was prepared by adding 40 mg of plant extract in 10 mL DMSO solution.

The antibacterial activity was determined by disc method where gentamycin was used as standard and distilled water as control. 1.5 mL of sample was added at different concentrations of 10, 20, 30 mg in the respective vials and sterilized discs were allowed to soak for an hour. Nutrient Agar media was poured into petri plates and allowed to solidify followed by inoculation of bacteria by spread plate method. The discs were removed from vials, completely drained of excess liquid and placed on the agar gently. Further, the petri plates were incubated at 37 °C for 24 hours. The zone of inhibition around each disk were then measured [24].

Antifungal Activity: The *in-vitro* antifungal activity test was conducted against *Aspergillus niger*. The sample was prepared by adding 40 mg of plant extract in 10 mL DMSO solution. The antifungal activity was determined by disc method where Fluconazole was used as standard and distilled water as control. 1.5 mL of sample was added at different concentrations of 10, 20, 30 mg in the respective vials and sterilized discs were allowed to soak for an hour.

Potato Dextrose Agar media was poured into petri plates and allowed to solidify followed by inoculation of fungi by spread plate method. The discs were removed from vials, completely drained of excess liquid and placed on the agar gently. Further, the petri plates were incubated at room temperature for 48 hours. The zone of inhibition around each disk were then measured [24].

RESULTS:

Organoleptic Evaluation:

TABLE 1: ORGANOLEPTIC EVALUATION OF *CYAMOPSIS TETRAGONOLOBA*

Sl. no.	Parameter	Observation
1	Color	Bright light green
2	Shape	Straight, erect, long tender pods
3	Texture	Slightly slimy soft
4	Taste	Tasteless
5	Odor	Mild, slightly grassy aroma

Physicochemical Evaluation:

TABLE 2: PHYSICOCHEMICAL EVALUATION OF *CYAMOPSIS TETRAGONOLOBA*

Sample	Total ash (%)		Acid insoluble ash (%)		Water soluble extract value (%)		Alcohol soluble extract value (%)	
	OV	LM	OV	LM	OV	LM	OV	LM
Cyamopsis tetragonoloba	2.486%	NA	3.962%	NA	45.21%	NA	23.22%	NA

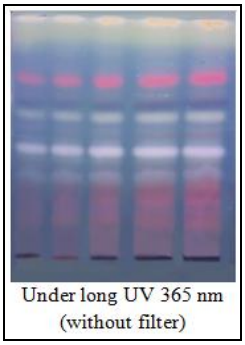
OV: Original value LM: Limit

Preliminary Phytochemical Evaluation:

TABLE 3: PRELIMINARY PHYTOCHEMICAL EVALUATION OF *CYAMOPSIS TETRAGONOLOBA*

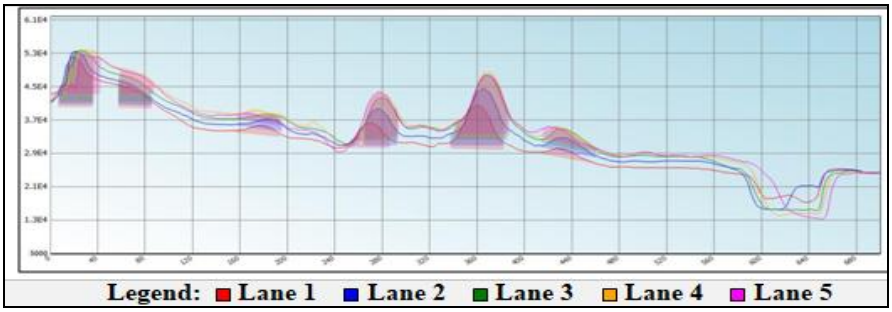
Sl. no.	Test	Result
1	Alkaloids	
	Dragendorff's test	Positive
	Mayer's test	Positive
2	Flavonoids- Shinoda test	Positive
3	Saponins- Froth test	Positive
4	Steroids	
	Liebermann-Burchard's Test	Positive
	Salkowski test	Positive
5	Triterpenes	
	Liebermann-Burchard's Test	Positive
	Salkowski test	Positive
6	Phenols- $FeCl_3$ test	Negative
7	Tannins	Negative

HPTLC Studies:



Under long UV 365 nm (without filter)

Analysis:



Lanes:

ID	Width	Bands	Volume
1	92	7	834.6
2	96	7	1099.46
3	102	7	1313.49
4	133	7	1666.85
5	118	7	1343.23

Microscopical Evaluation:

Powder Microscopy of *Cyamopsis tetragonoloba:*

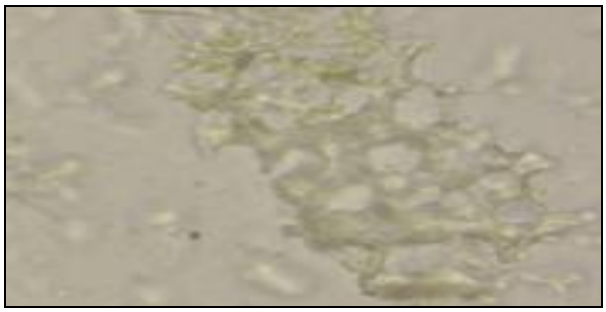


FIG. 1: GROUP OF PARENCHYMA CELLS

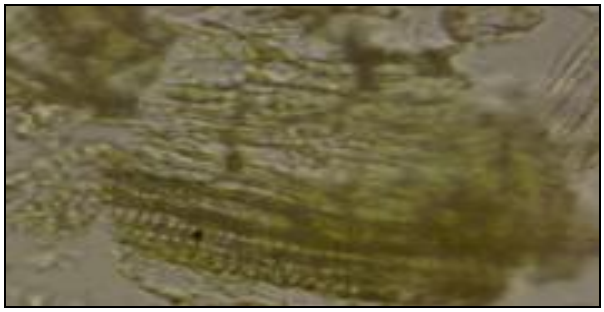


FIG. 2: EPIDERMAL CELL WITH STOMATA AND THICKENED HELICAL XYLEM VESSEL

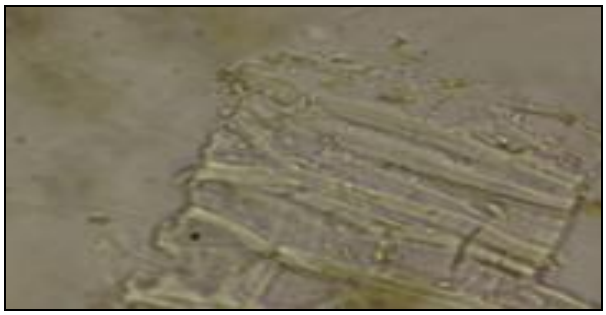


FIG. 3: EPIDERMAL CELLS

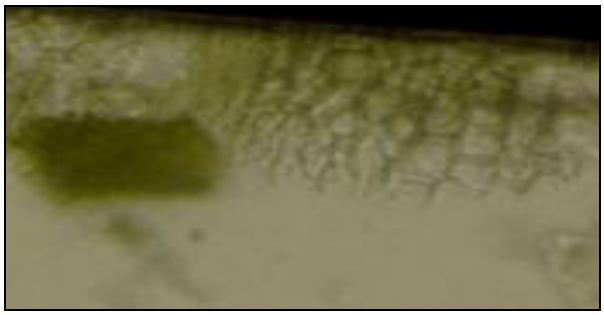


FIG. 4: EPIDERMAL CELLS AND MACROSCLEREIDS

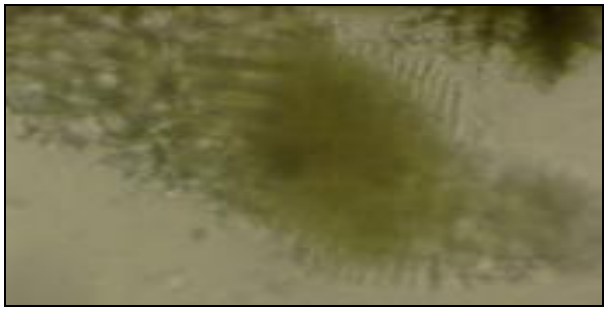


FIG. 5: MACROSCLEREIDS

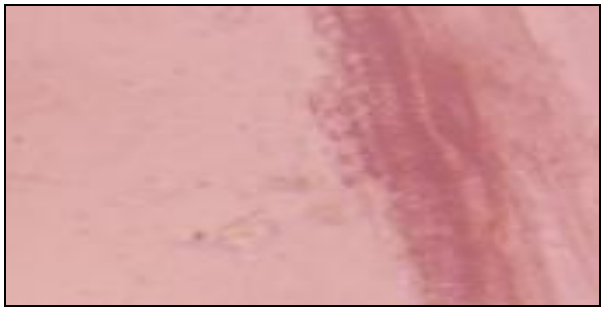


FIG. 6: THICKENED HELICAL XYLEM VESSEL

FIG. 7: SINGLE FIBRE

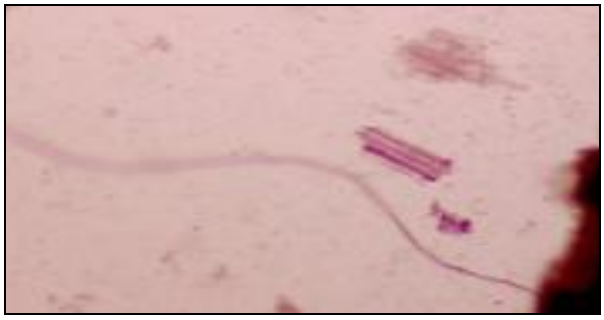


FIG. 8: XYLEM VESSEL

Fluorescence Analysis:

TABLE 4: FLUORESCENCE ANALYSIS OF *CYAMOPSIS TETRAGONOLOBA*

Sl. no.	Reagent added	Color
1	No reagent (raw sample)	Blue
2	Water	Creamy white
3	Conc. HNO_3	Pale yellowish-brown
4	Conc. H_2SO_4	Pale yellow
5	Dil. HCl	Yellow

6	Methanol	Bright pink
7	Petroleum ether	Blue
8	Hexane	Blue
9	Chloroform	Pink
10	Ethanol	Pink
11	Nitrocellulose	Bright pink

Microbial Limit Test:

TABLE 5: MICROBIAL LIMIT TEST OF *CYAMOPSIS TETRAGONOLOBA*

Sl. no.	Dilution	No. of colonies	CFU
1	control	No growth	0
2	10^{-1}	29	$29*10^{-1}$
3	10^{-2}	21	$21*10^{-2}$
4	10^{-3}	14	$14*10^{-3}$
5	10^{-4}	5	$5*10^{-4}$

CFU: Colony Forming Units

Microbial Activity:

Antibacterial Activity:

TABLE 6: ANTIBACTERIAL ACTIVITY OF *CYAMOPSIS TETRAGONOLOBA* AGAINST *BACILLUS CEREUS* AND *ESCHERICHIA COLI*

Sl. no.	Concentration of Antibiotic	Zone of inhibition (cm)	
		Against *B. cereus*	Against *E. coli*
1	Standard- Gentamycin	2.4 cm	1.0 cm
2	10 µL	1.0 cm	1.2 cm
3	20 µL	1.0 cm	1.0 cm
4	30 µL	1.0 cm	1.0 cm

Antifungal Activity:

TABLE 7: ANTIFUNGAL ACTIVITY OF *CYAMOPSIS TETRAGONOLOBA* AGAINST *ASPERGILLUS NIGER*

Sl. no.	Concentration of antibiotic	Zone of inhibition (cm)
1	Standard- Fluconazole	1.5 cm
2	10 µL	1.6 cm
3	20 µL	1.5 cm
4	30 µL	1.5 cm

DISCUSSION: The plant under study *Cyamopsis tetragonoloba*, was identified and authenticated as per the study requirements. The organoleptic and physicochemical studies conform with the limits mentioned in Ayurvedic Pharmacopoeia of India (API).

The preliminary phytochemical studies of methanolic extracts of *C. tetragonoloba* showed varied results except phenols and tannins, all other group of chemicals viz., alkaloids, flavonoids, saponins, steroids and triterpenes were present. As far as HPTLC fingerprinting profile is concerned, 7 bands were observed in all 5 lanes of 2 µL, 4 µL, 6 µL, 8 µL and 10 µL at Rf values 0.96, 0.90, 0.75, 0.63, 0.60, 0.48, 0.39. The sample exhibited various normal and fluorescent colours when they were treated with water, different types of acids, and other various solvent systems. In the microbial limit test, the colony-forming unit (CFU) both in bacteria and fungus, the ranges were well within the limits as mentioned in API. Having a large group of chemicals, *C. tetragonoloba* exhibited moderate antimicrobial activity.

Fluorescence studies of the sample powder on treating with various solvents and with nitrocellulose, indicated different colors when observed under visible and UV light (365 nm). The activity of pulp of *C. tetragonoloba* is the same in 10 µL against *Bacillus cereus* as that of standard Gentamycin, whereas it is negligibly more than standard against *E. coli* in the same concentration. In all three concentrations such that 10 µL, 20 µL,

and 30 µL of *C. tetragonoloba*, the activity is moderate against *Aspergillus niger*, almost similar to that of standard Fluconazole. The antimicrobial activity of *C. tetragonoloba* may be attributed to the group of chemicals present in plant parts under study. The HPTLC fingerprint profile of methanolic extract of this plant showed 7 bands each in 5 lanes of increasing concentration and a pattern unique to *C. tetragonoloba* and it can be used as quality standard method for identification and authentication.

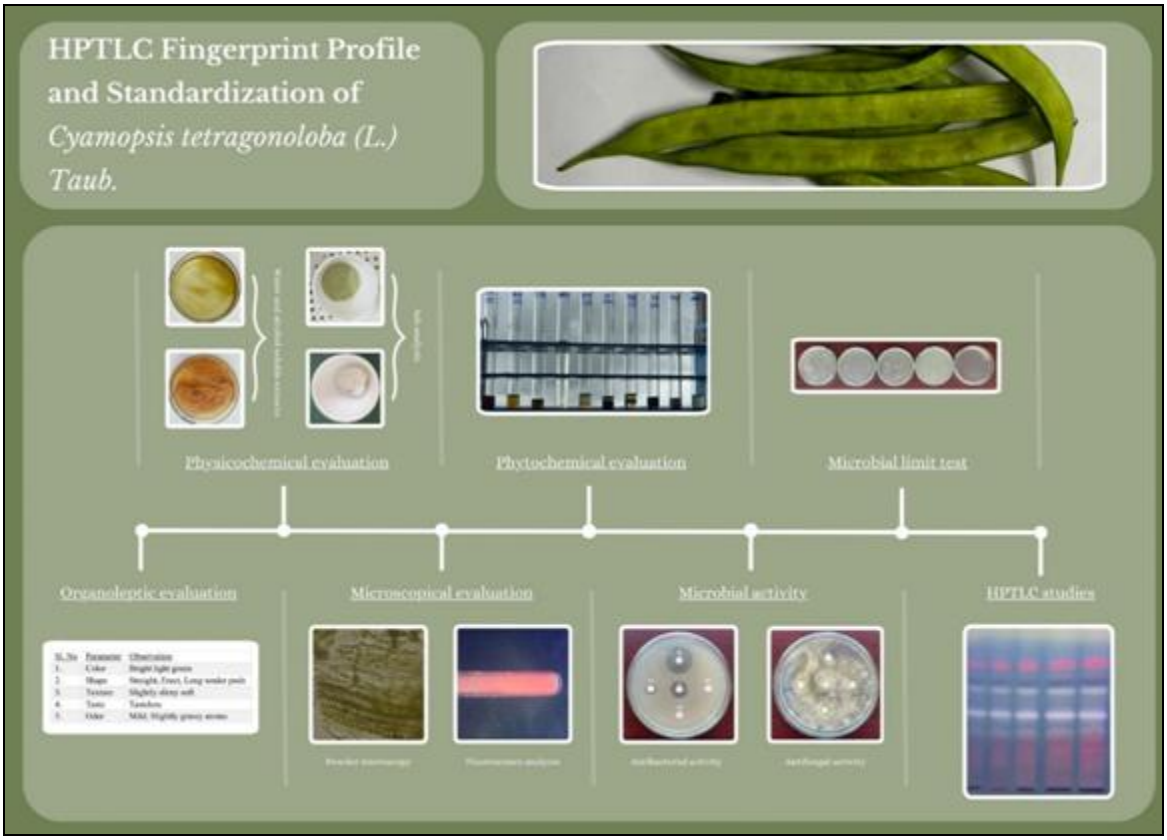


FIG. 9: HPTLC FINGERPRINT PROFILE OF *CYAMOPSIS TETRAGONOLOBA*

CONCLUSION: From the above study, it can be concluded that pharmacognostic and phytochemical evaluation will possibly help as a valuable resource for the identification, authentication, and preparation of the monograph of *Cyamopsis tetragonoloba*. The present work was embraced with a perspective of setting down a benchmark which could be valuable in recognizing the authenticity of therapeutically important medicinal plants. Microscopical studies have demonstrated the presence of parenchyma cells, macrosclereids, thickened xylem vessel, fibres, and epidermal cells.

The phytochemical investigation showed the presence of alkaloids, flavonoids, saponins, steroids and triterpenes. Thus, obtained results can be utilized for the quality control of the crude drug/ drugs. This type of study helps standardize the drugs and can be used to differentiate closely related or allied species. The HPTLC results provide standard fingerprints that can be used as a reference result for the identification and QC of the drug. This can also be used as a good tool in preparing the monograph. The various physicochemical parameters were established which are also important in analyzing adulteration and mishandling of the crude drug. Further spectral studies and *in-vivo* studies can be done to know their exact chemical composition and therapeutic efficacy.

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CONFLICT OF INTEREST: None

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